15. Genetic inborn error of metabolism provides unique window into molecular mechanisms underlying taurine therapy

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Abstract. Taurine has found empirical uses in the treatment of several disorders and is active as an anti-oxidant and in the control of cellular calcium homeostasis, but its therapeutic mechanism of action remains to be defined. Rare inborn errors of metabolism have historically proved to be invaluable tools in dissecting mechanisms of disease, and often have provided the first clues to the molecules and pathways involved in pathogenic processes underlying common diseases mirrored by these rare genetic syndromes. This, in turn, has provided unique opportunities for the discovery of novel therapeutics. Succinic semialdehyde dehydrogenase (SSADH) deficiency, a rare neurometabolic disorder, seems positioned to play such a role for taurine. A male child, product of a consanguineous marriage, was referred to us with a diagnosis of congenital hypotonia, ataxia and global developmental delay. Abnormal signals in the globus pallidi and subcortical white matter on magnetic resonance imaging (MRI) suggested a mitochondrial disease. Urine organic acid analysis revealed a markedly elevated γ-hydroxybutyrate

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(GHB) and related metabolites, confirming the diagnosis of SSADH deficiency, and subsequent genotyping studies proved this diagnosis. Taurine had recently proven effective in a lethal knock-out mouse model of this condition. In the absence of alternative effective therapies, the family began treatment with empirically increased doses of taurine. This resulted in marked clinical improvement and reversal of MRI-documented lesions after 12 months of therapy. This successful first trial of taurine as a therapeutic agent for treating human SSADH deficiency warrants systematic evaluation in controlled clinical trials, and potentially points to molecular targets involved in other taurine-responsive disorders.

### Abbreviations

Aldh5a1 -/-, SSADH deficient knock out null mice; CNS, central nervous system; DHHA, 4,5-dihydroxyhexanoic acid; GABA, γ-amino-butyric acid; GABA-T, γ-amino-butyric acid-transaminase; GAD, glutamic acid decarboxylase; GHB, γ-hydroxy-butyrate; HNE, 4-hydroxy-trans-2-nonenal; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, N-acetyl aspartate; SSA, succinate semialdehyde; SSADH, succinate semialdehyde dehydrogenase; TCA, Krebs tricarboxylic acid cycle.

### 1.1. Introduction

Taurine is an endogenous non-protein sulfonic amino acid synthesized in the liver from cysteine and also derived from breast milk and animal proteins in the diet (Shao and Hathcock, 2008). It is one of the most abundant amino acids in the body and is found in most tissues and biological fluids. It occurs at its highest concentrations, however, in excitable tissues such as nerve and muscle. Taurine has found empirical uses in the treatment of several disorders, and published human studies have reported minimal adverse effects at dosages up to 10 g/d, more than twenty times the estimated US adult consumption in foods (Shao and Hathcock, 2008). Taurine is active as an anti-oxidant and in the control of cellular calcium homeostasis (El Idrissi, 2008), but its therapeutic mechanism of action remains to be defined.

Rare inborn errors of metabolism have historically proven to be invaluable tools in dissecting mechanisms of disease, and often have provided the first clues to the molecules and pathways involved in pathogenic processes underlying common diseases mirrored by these rare genetic syndromes. This, in turn, has provided unique opportunities for the discovery of novel therapeutics with potentially broad utility (Gargus, 2005; 2009). Succinic semialdehyde dehydrogenase (SSADH) deficiency, just such a rare inborn error, holds the potential to prove such a touchstone for taurine’s therapeutic actions.
SSADH deficiency (OMIM # 271980) is a neurometabolic disorder now with over 400 reported cases. SSADH is a NAD$^+$-dependent mitochondrial matrix enzyme (EC 1.2.1.24, Gene ID: 7915) encoded by the gene ALDH5A1. This enzyme is involved in the γ-amino-butyric acid (GABA) shunt located in the mitochondria (Figure 1). GABA is the major inhibitory neurotransmitter and it is derived by decarboxylation of glutamate---the major excitatory neurotransmitter---by glutamic acid decarboxylase (GAD). This GABA-glutamate shuttle maintains the inhibitory-excitatory balance in the central nervous system (CNS). GABA is deaminated to succinic semialdehyde (SSA) by GABA transaminase (GABA-T), consuming alpha ketoglutarate from the Krebs tricarboxylic acid (TCA) cycle and regenerating glutamate in the process. SSA is then oxidized by SSADH to succinate, which enters the TCA cycle for energy production via oxidative phosphorylation. A deficiency of SSADH leads to accumulation of SSA, which is reversibly converted to γ-hydroxy-butyric acid (GHB), the major neurotoxin in this disorder. This compound, endogenously massively over-produced in patients with SSADH deficiency, is itself also used as a recreational drug that has come to be associated with “date rape” because of its rapid onset of action (Gropman, 2003). Elevation of GHB in urine organic acid analysis by isotope dilution gas chromatography-mass spectroscopy is the biochemical hallmark for diagnosis of SSADH deficiency (Pearl et al., 2009).

Clinical features of SSADH deficiency are primarily neurological, but nonspecific. The age of presentation can range from infancy to the late 20s. Non-progressive ataxia, seizures, motor delay, hypotonia, speech delay, hyporeflexia, autistic features, behavioral hyperactivity, aggressiveness and sleep disturbances with reduced REM stage have all been reported (Pearl et al., 2009). Neonatal presentations can include prematurity, hypoglycemia and respiratory distress (Gordon, 2004). Increased T2-weighted signal abnormalities in the globus pallidus and subcortical white matter are the most common abnormalities reported on MRI in SSADH deficiency (Gibson, 2005; Pearl et al., 2003). Discoloration of the globus pallidus was recently demonstrated on post mortem neuropathology (Knerr et al., 2008), consistent with the MRI findings.

Most current treatments for SSADH deficiency are symptomatic. Methylphenidate has been employed for attention deficit or hyperkinesis; and benzodiazepines and selective serotonin re-uptake inhibitors for anxiety-related behavior (Gropman, 2003, Gibson, 2005). Various anticonvulsants have been tried for seizure control, some ineffectively (phenytoin and phenobarbital) (Gibson et al., 1997; Gupta et al., 2003) and others with variable success (carbamazepine, lamotrigine, and most widely used vigabatrin) (Gibson, 2005).
In this paper we discuss our experience with a case of SSADH deficiency treated with taurine, and our encouraging results. Empirical treatment with taurine in our patient resulted in marked clinical improvement and reversal of MRI-documented lesions after 12 months of therapy and has sustained continued improvement now for over 4 years. To our knowledge, ours is the first case describing the use of taurine in the treatment of SSADH deficiency (Pearl et al., 2009).

1.2. Clinical experience with taurine therapy in SSADH deficiency

Our patient is a 7 8/12 year old male born at full term via caesarian section secondary to macrocephaly. There were no prenatal, perinatal or neonatal complications. He was the second pregnancy of healthy Hispanic parents, with the first being an early miscarriage. The third pregnancy was a 5 ½ year old healthy female. There is history of consanguinity, with the patient’s maternal grandmother and paternal great-grandmother being sisters. The family history was otherwise unremarkable. He was first seen by neurology at two years of age and diagnosed with global developmental delay, central hypotonia and ataxia. He did not walk independently or use single words until about age two. There was no history of loss of milestones. Brain MRI performed at 2 4/12 years of age revealed transient restricted diffusion (felt to represent reversible cellular edema), and bilateral symmetrically enhanced T2-weighted signals in the globus pallidi with periatrial insular cortex and central periventricular white matter changes, highly suggestive of a mitochondrial disease. Magnetic resonance spectroscopy (MRS) revealed an inverted creatine/choline ratio and elevated N-acetyl aspartate (NAA) levels, both abnormal signals but of unknown significance. He was referred to us at 2 8/12 years of age for diagnosis based upon his worsening ataxia, deteriorating clinical condition and the abnormal imaging findings. In that evaluation his plasma electrolytes, lactate, pyruvate, acyl carnitine profile and ammonia were all normal. Urine organic acid analysis, however, revealed a markedly elevated GHB and related metabolites, establishing the diagnosis of SSADH deficiency. Much later he was genotyped and shown to be a compound heterozygote for two pathogenic alleles of Aldh5a1. In exon 3 he carried the missense c.608C>T allele that results in substitution of a leucine for a proline, and in exon 6 he carried the c.858delT frame shift allele.

At the time of diagnosis we reviewed with the parents that in the Aldh5a1−/− knock-out mouse model of the disease, supplementation with the amino acid taurine had recently been shown to be an effective treatment
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(Hogema et al., 2001), and that human studies had shown low toxicity of this natural product that was routinely sold as a health food. We recommended that he be started on vitamin B6, a known co-factor of the affected enzyme, and the parents elected to start him additionally on taurine supplements based upon the promising response in the mouse model and good safety profile of taurine. We agreed to monitor his GHB and taurine levels as his oral dose of taurine was increased empirically. By three months of treatment, he was taking 2 g taurine orally, twice daily (~200 mg/kg.d) without problems. At this dose his plasma taurine levels ranged from 2-8 fold normal. He was maintained at this dose since the family noted insomnia at higher doses. At this point his ataxic gait as well as playground coordination and energy levels were markedly improved. His clear resolution of ataxia is particularly worth noting because a large questionnaire-based study involving 60 individuals with SSADH did not find any resolution or improvement of ataxia with age (Pearl et al., 2009). By nine months of treatment, his teachers reported markedly improved behavior, peer interactions, level of activity and coordination. California Children’s Services evaluation at that time determined that he no longer qualified for therapy services [They were persuaded to continue!]. At 12 months of treatment, a repeat MRI was obtained and officially read as “normal”. Careful comparison with the prior study showed that the predominating globus pallidus lesion of restricted diffusion had completely reversed and was now normal. A very small residual T2-signal abnormality remained. No correlation was found between urine GHB levels and severity of clinical symptoms in our patient, compatible with other reports (Gibson et al., 1997). Additionally, there was no correlation between plasma taurine levels and urine GHB levels. At 5 2/12 years of age, the patient had his first seizure episode and he continues to have occasional seizures. Seizures are seen in 50% of patients with SSADH deficiency (Pearl et al., 2009). His seizures usually last for 30 seconds to a minute and do not require medications to be broken. They involve eye rolling and loss of consciousness, but no tonic-clonic activity. They are currently being treated with Levetiracetam.

1.3. Discussion

Defining the pathogenesis of a complex neurogenetic or metabolic syndrome remains a major challenge in medicine. In this post-genomic era of molecular medicine, a strong paradigm is initiated by the definition of the genes that contribute to pathogenesis. This in turn leads to recognition of the physiological pathways underpinning the pathogenic mechanism. Genome-wide association studies (GWAS) provide the most recent wave of such
insights into common diseases, but historically the rare inborn errors of metabolism---simple Mendelian genetic diseases---have provided the most powerful window into normal physiology and the pathophysiology underlying disease phenotypes (Gargus, 2005; 2009). The true payoff of such molecular analyses is the identification of a molecular target that has the potential to lead to a novel therapeutic that will treat the disease phenotype. To a large extent this “rational drug design” has supplanted serendipity in the discovery of novel therapeutics.

The \textit{Aldh5a1}^{−/−} knock-out mice have already proven invaluable in understanding SSADH deficiency in man (Pearl et al., 2009; Hogema et al., 2001), and should begin to open the vista to other disorders along this mitochondrial GABA-shunt pathway. The impressive responsiveness of this molecularly-defined disease of mouse and man to taurine therapy, in a converse application of the discovery paradigm discussed above, may implicate this same pathogenic pathway in \textit{other diverse} disorders already showing taurine responsiveness.

Since an early hallmark of SSADH deficiency, now well documented, is a high level of GABA and GHB in the CNS, these two neurochemicals are promising candidates to evaluate for a role in producing the taurine-responsive pathophysiology of SSADH deficiency. Further downstream effects of chronically elevated CNS GABA levels are alterations in GABA receptor signaling and downregulation of the \textit{GABA}_A and \textit{GABA}_B receptors in the CNS (Gibson, 2005). While thus far signaling studies remain confined to the mouse model, discussed below, receptor downregulation has already been directly observed in human SSADH deficiency (Pearl et al., 2009). Additionally, GABA, but not GHB, activates the \textit{GABA}_A receptor. This receptor inhibits 3-beta-hydroxysteroid dehydrogenase, thus reducing the synthesis of neuroactive steroids like allopregnanolone, pregnenolone and progesterone. They are known to have anticonvulsant effects by binding to the \textit{GABA}_A receptor (Gee et al., 1995), and are found at reduced levels in the murine model (Gupta et al., 2003).

Preclinical therapeutic trials have made use of the lethal seizure phenotype of the \textit{Aldh5a1}^{−/−} mice, and initial treatment strategies were anticonvulsants targeted at the GABA shunt pathway directly perturbed by this mutation. Phenytoin and phenobarbital were ineffective (Hogema et al., 2001). Vigabatrin (gamma-vinyl-GABA: Sabil), an irreversible GABA transaminase inhibitor (Figure 1), was perhaps the most obvious choice in anticonvulsants, and the most efficacious in the mouse model (Hogema et al., 2001).

This structural GABA analogue (Gropman, 2003) prevents conversion of GABA to SSA by inhibiting GABA-T activity, leading to an increase in GABA and a decrease in GHB. In SSADH deficient individuals, vigabatrin
Figure 1

was shown to alleviate hyperactivity and behavioral problems, increase alertness and attention span, and to normalize EEG recordings with reduced seizures and improved ataxia (Ergezinger et al., 2003). However results were inconsistent, with only one third of the patients responding to this drug (Gibson et al., 1997) and seizure control diminishing with chronic use. Since vigabatrin further elevates GABA in these patients (who already have a two-fold elevated level) its chronic use could potentially exacerbate the downstream effect of GABA on receptor downregulation, and by this means produce the paradoxical loss of GABA inhibitory effects on CNS action potentials. This in turn could lead to the enhanced excitability and increased seizures seen with chronic use. Chronic use of vigabatrin also can lead to a restriction of visual fields and a panoply of other side effects that limit its safety profile such that while it is now approved by the Food and Drug Administration (FDA) with signed informed consent, its distribution is strictly regulated.

While it is apparent that the major symptoms of SSADH deficiency are neurological, understanding the pathology arising from elevated GABA and GHB is further complicated by the fact that SSADH is active in multiple organs, and that its deficiency in the liver, where hepatic production of GHB occurs, adds a new potential pathway to pathogenesis (Gupta et al., 2004). This raises the possibility that SSADH deficiency in the liver is an important source of GHB accumulation in the blood. Since GHB readily crosses the blood-brain
barrier, it could thereby be responsible for causing the perturbations in the brain neurochemistry, and the subsequent neurological phenotype of the disease. In fact, despite achieving the expected rise in CNS GABA levels in murine and human SSADH deficiency, vigabatrin does not effectively reduce the brain GHB levels (Gupta et al., 2002). The drug is less effective in the liver than the brain at limiting GHB production, and likely resupply of GHB from the liver to the brain contributes to vigabatrin’s limited efficacy (Gupta et al., 2002; 2003; Ergezinger et al., 2003). Given the apparent importance of suppressing the GHB levels produced by the liver, gene therapy using a hepatotropic adenoviral vector containing human SSADH cDNA was tried in the Aldh5a1−/− mouse model (Gupta et al., 2004). It was reported that with only a modest expression of recombinant SSADH enzyme in the liver, the peripheral GHB levels dropped by over 70% and CNS GHB by half, resulting in more activity, more responsiveness to their surroundings, and a significant prolongation of lifespan (Gupta et al., 2004; Gibson, 2005). Besides presenting a potential therapy, this finding minimally puts a sharper focus on the actions of GHB in the disease pathogenesis, and the need to focus understanding therapeutic responses in the context of this neurochemical.

GHB is a monocarboxylic short-chain fatty acid that occurs naturally in the brain and functions as a neurotransmitter at GHB receptors (GHBR) and other neuroreceptors, including the GABA_B (but not GABA_A) receptors, the N-methyl-D-aspartate glutamate receptors (NMDAR), and the opioid receptors (Gropman, 2003; Knerr et al., 2007). It can produce absence seizures, amnesia, coma and death (Knerr et al., 2007). Trials of selective receptor inhibitors in the murine model of SSADH deficiency further support the importance of GHB in pathogenesis. The GHB receptor antagonist, NCS 382 [6, 7, 8, 9-tetrahydro-5-(H) benzocycloheptane-5-ol-4-ylideneacetic acid] led to a 60% survival rate of Aldh5a1−/− mice, comparable to that of vigabatrin (Hogema et al., 2001; Gupta et al., 2003; Gibson, 2005). On the other hand, the GABA_B receptor antagonist CGP 35348 [3-aminopropyl (diethoxymethyl)-phosphinic acid], while effective in suppressing absence seizures, exacerbated convulsive seizures, and achieved a more modest 25% survival (Hogema et al., 2001).

GHB is degraded via fatty acid oxidation and reduces neuronal glucose utilization. These metabolic effects, and the prominence of seizures in SSADH deficient patients and mice, makes reasonable trials of diet therapy using the ketogenic diet, long an empirically-useful mainstay in the management of intractable seizures. In the Aldh5a1−/− mice the ketogenic diet was found to resolve ataxia, normalize EEG recordings, delay onset of status epilepticus, encourage weight gain and prolong the lifespan four-fold in the mice (Nylen et al., 2008). The ketogenic diet provided an alternative source
of energy, likely aiding weight gain. At a biochemical level, this diet was shown to repopulate mitochondria in the hippocampus in Aldh5a1−/− mice (a region of the brain prominently affected in SSADH deficiency) and restore ATP to normal levels (Nylen et al., 2009). At a molecular level, there was evidence that this diet salvages activity of inhibitory GABA<sub>A</sub> receptor-gated chloride channels, preventing seizures (Nylen et al., 2008). However, efficacy of this diet in SSADH deficient patients is limited and untested in controlled trials.

More recent data from the Aldh5a1−/− mouse model has revealed that the biochemical derangements in SSADH deficiency go well beyond the metabolites of the GABA shunt, many culminating in increased oxidative stress (Latini et al., 2007). Interestingly, at least in plants, the GABA shunt has been shown to be important in maintaining redox equilibrium, with a compromised GABA shunt or high levels of GHB itself causing reactive oxygen intermediates (ROI) and H<sub>2</sub>O<sub>2</sub> accumulation (Bouché et al., 2003; Fait et al., 2005). GHB decreases dopamine synthesis and release, and increases turnover of biogenic amines and catecholamines (Gupta et al., 2003). This last action can potentially add to the oxidative stress since monoamine oxidase, the enzyme involved in dopamine metabolism, generates H<sub>2</sub>O<sub>2</sub>. Also, catecholamines undergo spontaneous oxidation, generating superoxide radicals (Gupta et al., 2003). GHB can be acted upon by D-2-hydroxyglutarate transhydrogenase converting it into SSA with stoichiometric conversion of alpha-ketoglutarate to D-2-hydroxyglutarate. This has been reported in the brains of both the Aldh5a1−/− mice and in SSADH patients. D-2-hydroxyglutarate activates glutamatergic receptors and induces oxidative stress (Gibson, 2005). Further hints of oxidative stress in the Aldh5a1−/− mice are decreased levels of the important antioxidant glutathione (Sauer et al., 2007) and elevation of alanine, suggestive of a lowered NADH/NAD<sup>+</sup> pool, and cystathionine (Gupta et al., 2003). Cystathionine synthesis requires cystathionine-beta-synthase, a heme protein known to be upregulated under conditions of oxidative stress (Gupta et al., 2003). Finally, 4,5-dihydroxyhexanoic acid (DHHA), an active respiratory chain inhibitor that forms from the condensation of accumulating SSA with acetyl-coenzyme A, has been documented to be elevated in the mouse model even with no alterations observed in the Krebs TCA intermediates (Gropman, 2003; Gupta et al., 2003; Gibson, 2005). It is also of note, particularly for interpreting taurine responses in other neurological disorders, that SSADH is the predominant enzyme in CNS mitochondria for oxidative detoxification of 4-hydroxy-trans-2-neoneal (HNE), a toxin known to cause oxidative damage in mitochondria, and reported to be elevated in Parkinson’s disease, Alzheimer’s disease and cerebral ischemia (Murphy et al., 2003). Furthermore, it is
intriguing that SSADH is a rapidly-evolving human gene, a signature of genes taking on special roles in modern man. The human polymorphic missense variant, c.538C, is rapidly replacing the ancestral primate c.538T allele in populations since their emergence from Africa (Leone et al., 2006).

It is in the context of this potential pathogenesis via oxidative stress that attention in SSADH deficiency in the mouse model came to be focused on taurine (2-aminoethanesulfonic acid). It was observed in the Aldh5a1+/− mice that the onset of status epilepticus correlated closely with the weaning period and those mice that suckled longer lived longer. Taurine, one of the most abundant amino acids in mammalian milk, and one with well documented antioxidant properties (Huxtable, 1992; Saransaari and Oja, 2000), was considered a potential constituent in the milk providing this protection (Hogema et al., 2001). Taurine is found at high concentrations in the developing hippocampus, a region showing gliosis in immunohistochemical studies of the Aldh5a1−/− mice (Gropman, 2003). Taurine is thought to play a role in the development and survival of neurons (Gropman, 2003). It is protective against seizure and ischemic induced injuries in both animals and humans (Gropman, 2003; Gupta et al., 2003). Also, it activates both GABA_A and GABA_B receptors, both integral in pathogenesis of SSADH deficiency (Gropman, 2003, Wang et al., 1998). Given these qualities, preclinical trials were undertaken in the Aldh5a1+/− mice. In these SSADH deficient mice, intraperitoneal administration of taurine at 250 mg/kg led to 56% survival, comparable to vigabatrin, and oral administration of taurine at 2000 mg/kg led to 38% survival (Hogema et al., 2001). Higher doses were toxic with lower survival rates (Gupta et al., 2002).

1.4. Conclusion

Based on the positive results in mice and the fact that taurine is commonly sold as an over the counter supplement with a well documented safety profile in humans at 10 g/day, and with no reported significant adverse effects at even higher doses (Shao and Hathcock, 2008), the parents of our patient elected to proceed with taurine supplementation with encouraging results as discussed above. Although encouraging, this is an isolated case. We conclude that although taurine is not a cure for SSADH deficiency, it does appear to have a role in the clinical management of patients with this condition and that a systematic evaluation of taurine as a therapeutic agent for treating SSADH deficiency using controlled and blinded clinical trials is warranted. It is also hopeful that its efficacy in this molecularly-defined disease will provide a touchstone for defining the mechanism of taurine’s efficacy more broadly.
1.5. References